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The Humoral Immune Response to Asaccharolytic *Eubacterium* Species in Periodontitis

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Asaccharolytic *Eubacterium* species are strongly associated with advanced periodontal disease. Raised systemic antibody levels to *Eubacterium brachy*, *E. nodatum* and *E. timidum* have been found in periodontitis patients compared to healthy controls using ELISA and RIA. This study compared antibody profiles in periodontitis patients and controls against oral asaccharolytic *Eubacterium* species by Western blotting. Whole-cell proteins from strains of *E. brachy*, *E. nodatum*, *E. timidum* and representative strains of five candidate species were separated using PhastSystem SDS–PAGE. The proteins were electroblotted onto nitrocellulose and probed with 23 sera from periodontitis patients and 23 from periodontally healthy age- and sex-matched controls. Antibodies were present to proteins of all strains except *E. nodatum* but there was no relationship between patterns of antigen recognition and periodontal status.

KEY WORDS—*Eubacterium*; Antibodies; Sera.

INTRODUCTION

The slow growth and oxygen sensitivity of the oral asaccharolytic *Eubacterium* species has hindered the determination of their significance in periodontal disease.[2] They are found in numbers in advanced periodontal disease and are only rarely detected in oral health.[4–7,9] There are currently three named species in the group: *Eubacterium brachy*, *E. nodatum* and *E. timidum*. However, a number of additional distinct taxa have been recognised.[4–7,9,12]

In addition to the numerical association noted above, antibody levels to *E. brachy*, *E. nodatum* and *E. timidum* have been shown to be raised in patients with periodontitis compared to controls.[3,8] *E. brachy*, in particular, exhibits an immunoreactive antigen of molecular weight 170 000 which has been suggested as a possible virulence factor.[10,11] In the study of other organisms, specific determination of the protein components reactive with patient antibodies has been shown to be useful in the characterisation of discriminatory markers, notably antibody to the 47-kDa protein of *Porphyromonas gingivalis* which is present in patients and absent in controls.[1]

The purpose of this study was to compare the immune response to the oral asaccharolytic *Eubacterium* species in patients with periodontal disease and healthy controls using Western blotting and immunostaining.

MATERIALS AND METHODS

The type strains of *E. brachy* (ATCC 33089), *E. nodatum* (ATCC 33099) and *E. timidum* (ATCC 33093) and strains W1365, W1471, 93G, 3D and 26 were grown in anaerobic conditions for 48 h on Fastidious Anaerobe Agar (FAA, Lab M, Bury, UK). Sample preparation was optimised to yield the maximum number of protein bands without overloading of the gel; method development has been described previously.[12] Briefly, organisms were harvested and diluted 1 in 15 (w/v) with sample-dissociating buffer containing 10 per cent SDS/25 per cent 2-β-mercaptoethanol, followed by vigorous shaking with glass beads and centrifugation (MicroSpin 12, Sorvall Instruments, DuPont,

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Table 1. Number of sera from periodontal disease patients (p, $n=23$) and periodontally healthy subjects (h, $n=23$) exhibiting antibodies to specified antigens from asaccharolytic *Eubacterium* species assessed by Western blotting (** – $P<0{\cdot}01$, Chi-squared analysis)

Antigen number	Strain number																			
	33093			3D			93G			26			1365			1471			33089		
	MW	p	h	MW	p	h	MW	p	h	MW	p	h	MW	p	h	MW	p	h	MW	p	h
1	260	1	3	>300	1	0	>300	9	14	>300	0	1	190	0	1	203	2	2	150	3	5
2	167	0	1	115	0	1	>300	6	4	>300	2	0	150	0	1	159	4	3	109	5	4
3	135	0	1	90	6	4	>300	6	3	150	5	3	80	1	0	128	5	5	87	13	15
4	115	2	2	65	5	3	280	8	11	92	4	3	67	1	0	99	5	5	71	5	5
5	96	2	3	45	2	2	216	7	8	76	2	2				73	1	0	59	1	1
6	60	1	0	37	1	1	173	4	2	65	1	4				16	18	15	46	0	1
7	15	1	6	25	3	3	142	6	4	51	1	0									
8				21	16	20	112	6	4	29	0	1									
9				14	0	2	78	5	7												
10							58	12	10												
11							45	2	2												
12							34	1	4												
13							30	4	4												
14							16	11	1**												

Stevenage, UK) at 13 000 *g* to provide a solubilised bacterial protein supernatant. Proteins were separated by SDS–PAGE with the PhastSystem (Pharmacia-LKB, Uppsala, Sweden) and 10–15 per cent gradient SDS–polyacrylamide gels. The separation programme was as previously described.[12] Immediately following separation the gels were PhastTransfer (Pharmacia-LKB) electroblotted onto nitrocellulose membranes. Sera were obtained from 23 patients with advanced, chronic and untreated periodontitis (minimum of six sites bleeding on probing and pocketing >4 mm) and 23 healthy age- and sex-matched controls (no bleeding on probing, no pockets >3 mm). The nitrocellulose blots were blocked against non-specific binding in 5 per cent bovine albumin, fraction *V* (BA) (BDH, Poole, Dorset, UK) in phosphate-buffered saline, pH 7·2 (PBS) for 1 h. The blots were incubated with agitation in 5 ml 1/20 sera in 1 per cent BA in PBS for 1 h and wre washed in 0·1 per cent Tween 20 in PBS for 4 × 5 min followed by 5 min in PBS. The serum dilution used was determined in pilot studies and was chosen to optimise antigen recognition without non-specific binding. Blots were incubated in 30 ml 1/1000 goat anti-human IgG alkaline phosphatase conjugate (Sigma Chemical Co., Poole, Dorset, UK) in 1 per cent BA in PBS for 1 h with agitation. The blots were again washed. Fast red TR salt (Sigma) in conjunction with naphthol AS . MX phosphate (Sigma) was used as substrate[8] allowing visualisation of red-stained antibody profiles. Antibody profiles were scanned in a Chromoscan 3 densitometer (Joyce-Loebl) in reflection mode using a 530 nm filter and an aperture of 0·05 × 1·5 mm. Peak heights >5 mm above background were considered to be significant and the number of sera giving peaks noted. The numbers of sera from periodontal disease and control patients exhibiting antibody to given antigens were compared by the Chi-squared test with Yates' correction.

RESULTS

The results are shown in Table 1. No antibodies were detectable to *E. nodatum* ATCC 33099 while 14 antigens from strain 93G were detected. The antibody response was extremely diverse with the majority of antigens being recognised by only a small proportion of sera. There was a significant difference in the numbers of sera reacting to the 16-kDa antigen of strain 93G but only half of the disease sera were positive. Given the number of comparisons performed here it is likely that this difference may have arisen by chance. There was therefore no difference between the patterns of immunodominant antigen recognition between patient and control.

DISCUSSION

Previous studies have shown raised antibodies to *E. brachy*, *E. nodatum* and *E. timidum* in patients with advanced periodontal disease compared to controls,[3,8] although by quantitative methods. This study has demonstrated no qualitative differences in systemic antibody response which could be used as markers of past or current disease. However, serum levels do not necessarily correlate with those found in gingival tissue which may be a more accurate reflection of the host response to periodontal bacteria.[3] It would have been desirable to compare the levels of response to each antigen in patients and controls. However, so few of either patients or controls demonstrated levels above threshold for any given antigen, this would have been statistically invalid.

The amount and diversity of the immune response to the oral asaccharolytic *Eubacterium* species suggest cross-reactivity with other antigens. *Eubacterium* species found in the normal flora of the gut might have induced the stimulation of antibodies capable of cross-reacting with oral species. Further studies could include the preadsorption of antibodies from sera using other *Eubacterium* species.

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